Membrane protein purification

Materials

- 200mM Tris-HCl pH8
- 200mM Tris HCl pH 8 + 10 mM $MgSO_4$
- Frenchpress

Preparation

1. Preculture the cells in 20 ml LB, let it grow overnight at 37°C
2. Culture the cells in 1L LB until OD_{600} = 0.6
3. Add 0,5mM IPTG to the culture (100ul 0,5M in 1L LB)
4. Incubate with IPTG 4 hours or overnight
5. Pellet the cells and use as small as possible 200mM Tris-HCl pH8 to solve the pellet
6. Store at -20°C

Method

7. Resuspend the pellet in 200mM Tris HCl pH 8 + $MgSO_4$; volume as small as possible
8. Immediately before disrupting in the Frenchpress, add a pinch of DNase and 1mM PMSF
9. Put the Frenchpress on 1,8 – 1,9 bar
10. Collect the broken cells
11. Centrifuge 15min at 7000 rpm, 4°C
12. Retain supernatant and put it in the special tubes
13. Centrifuge 1 hour at 40,000 rpm, 4°C
14. Solve the pellet (consisting of membranes) in 200mM Tris-HCl pH8 and store in -20°C